THE BEHAVIOR OF THE CHROMOSOMES IN THE SPORE MOTHER-CELLS OF HIGHER PLANTS AND THE HOMOLOGY OF THE POLLEN AND EMBRYO-SAC MOTHER-CELLS.

DAVID M. MOTTIER.

(WITH PLATES XI–XIV.)

THERE is probably no other problem which has presented greater difficulties to the botanical cytologist than the behavior of the chromosomes during the two successive nuclear divisions in the spore mother-cells of the higher plants. This is at once apparent, even to those who are not specialists in this field, from the different interpretations which have been advanced from time to time, and from the various and contradictory doctrines that are still held by different investigators.

From the zoological side, we are indebted to Flemming and his students for the first correct interpretation of the behavior of the chromosomes in the two successive nuclear divisions in the spermatocyte of Salamandra.

Flemming designated the first of these divisions as "heterotypic" and the second as "homotypic." The first division in Salamandra is characterized by a long period of growth of the cell and nucleus during the prophase, the appearance of the reduced number of chromosomes and their double longitudinal splitting; the first longitudinal fission taking place during the prophase and the second during the metaphase or anaphase.

The striking parallel between the first two mitoses in the micro- and macrospore mother-cells of higher plants and those of the spermatocyte of Salamandra has frequently been pointed out, and the terms heterotypic and homotypic have been appropriately applied to them.

Recent investigations have shown that in the spore mother-cells of certain higher plants the first mitosis is especially characterized also by a long period of growth during the pro-

phase, the first appearance of the reduced number of the chromosomes, and the double longitudinal splitting of the latter.

It is chiefly upon the behavior of the chromosomes during these divisions that the great diversity of opinion rests, and this is not surprising when one realizes the complexity of the problem and the difficulties attending its investigation. However, in certain plants the double character of the daughter segments during the anaphase of the first nuclear division in the pollen mother-cell is so evident that one is surprised that it has been overlooked for so long a time and by so many observers. In all probability, this phenomenon may have been observed by earlier investigators, but as its significance was not understood the double nature of the daughter chromosomes may have been considered more apparent than real.

Heuser ('84) seems to have been the first to call attention to the double character of the daughter chromosomes in the diaster stage of *Tradescantia virginica*, but he interpreted the separation of the daughter segments during metakinesis of the first mitosis as a transverse division.

In 1895 Strasburger, basing his conclusions largely upon a study of Larix, properly explained the V-shaped daughter chromosomes, which so frequently appear during the anaphase of the first division in the spore mother-cell, as the result of a second longitudinal splitting taking place at right angles to the first. Each V-shaped element, therefore, represents two granddaughter chromosomes which remain attached at the ends to which the spindle fibers are fastened, while the opposite ends diverge, forming the V.

Two years later, Strasburger together with the writer abandoned the doctrine of the double longitudinal splitting, and the V- and U-shaped elements were explained as the result of a folding together of the chromosomes, or as a bending during metakinesis or previously, a view which had been held by other observers. Our conclusions were based largely upon the ring- or loop-shaped chromosomes which often occur in other plants, such as Podophyllum and Tradescantia, and upon phenomena observed in the second mitosis of the embryo-sac of *Lilium*

Martagon. In one respect we were correct, for in many cases the V- or U-shaped chromosomes are produced by a bending, either before or during the anaphase, but the fact that the V's and U's are double was overlooked.

Guignard ('99) found that in *Naias major* a second longitudinal fission occurs during metakinesis of the first nuclear division of the pollen mother-cell, and that each retreating daughter chromosome is composed of two rod-shaped granddaughter segments.

Grégoire ('99), in the same year, observed the same to be true for Lilium and Fritillaria.

Strasburger (1900), upon reinvestigating the subject in several different genera and species, found also that his explanation of 1895 was the correct one; for in all plants examined there occur during the first mitosis in the pollen mother-cell two longitudinal divisions of the chromatin, the first occurring, as the writer had very clearly shown, during the early prophase, the second during metakinesis, or while the daughter chromosomes are on their way to the poles. The second longitudinal splitting is a preparation for the second mitosis, which in Lilium does not pass completely into the structure known as the resting stage.

Since the appearance of the publications above cited, I have taken up the problem again, and have followed carefully both mitoses in the pollen mother-cells of *Lilium Martagon*, *L. candidum*, *Podophyllum peltatum*, and *Tradescantia virginica*, together with the corresponding nuclear divisions in the embryo-sac mother-cell of *Lilium Martagon*.

As regards the pollen mother-cell, I am able to confirm the results of Strasburger and the two French observers, namely, that during the first mitosis two longitudinal divisions of the chromosomes take place. I hope, however, to present more clearly certain details of the second mitosis in the pollen mother-cell, together with certain facts which have hitherto been too lightly touched upon, and add also the evidence which the embryo-sac contributes to the problem.

The earlier prophase of karyokinesis has been described in

minute detail, and I have nothing to add to my former account of the process (Mottier, '97). As soon as the chromatin spirem, which has split longitudinally, has segmented into the chromosomes, each chromosome consists in Lilium of two rather long daughter segments. These segments may be variously oriented toward each other even in the same nucleus. Sometimes they may be separated entirely from each other, being connected only by delicate threads; but, as is generally the case, they are more or less closely applied and twisted upon each other (*fig. 1, a*). Again, they may be attached to each other near the middle or at one or both ends. In the latter case, the free ends may diverge variously (*fig. 1, b, c*). It frequently occurs that the opposite ends adhere, while the middle portion bends outward to form loops or rings (*fig. 2, b*). In the majority of instances, it is clear that the daughter chromosomes are twisted upon each other, and to this fact is due many of the peculiar forms to be observed in the spindle stage.

Between the time of the segmentation of the chromatin spirem and the mature spindle, the chromosomes contract somewhat, becoming thereby shorter and thicker. They may lie distributed along the nuclear membrane or irregularly scattered in the nuclear cavity. At this stage, however, we frequently find the daughter segments so oriented toward each other as to present the various other forms often observed in the nuclear plate, such as loops, rings, X's, Y's, etc. With the development of the spindle, which occurs in precisely the manner described in my earlier paper (Mottier, '97), the chromosomes are gradually oriented in the nuclear plate (*fig. 3*). So far as can be determined with absolute certainty, each chromosome, up to the stage of the mature spindle, is composed only of two daughter segments, although in some cases a trace of the second longitudinal fission may be seen in the segments. It was this phenomenon that strengthened my former view that the chromosomes folded upon themselves during the development of the spindle.

As has been shown by numerous observers, the chromosomes arranged in the nuclear plate present various forms and various

modes of attachment to the spindle fibers. In Lilium the most frequently occurring form of chromosome at this stage is that of the two segments rather closely applied and twisted upon each other. They are attached to the spindle at the end, and stand radially to its long axis (*fig.* 2, *a*). The segments are frequently straight and not twisted upon each other.

Numerous instances are met with in which the daughter segments remain adhering at both ends, but separate near the middle, so that a ring or loop results (*fig. 2*, *b*). The segments may separate at one end, when an open ring or loop is formed. Cases are not infrequently observed in which the segments of the ring-shaped chromosomes bend near the middle so that the free ends are brought nearer together, and we have a ring or loop partly folded upon itself. This is true in a measure in *fig. 2*, *b*. The ring- or loop-shaped chromosome is attached to the spindle near one end of the daughter segments, or at a point midway between the ends, rarely at the ends; although such a mode of fastening was occasionally observed. As a rule, however, the rings or loops are placed tangentially upon the spindle.

When the daughter segments diverge at one end but remain closely applied for the remainder of their length, a Y-shaped chromosome results (*fig. 2*, *h*), which is fastened to the spindle as indicated in the figure. An X-shaped chromosome is formed when the segments lie variously crossed with one or both ends diverging (*fig. 2*, *f*). X-shaped chromosomes are not rare in which the segments are so closely applied at one end as to give the impression of a loop formed by the chromosome folding in such a way that the opposite ends are brought together (*fig. 2*, *g*). In fact all intermediate forms are found between that of *f* and *g*. Finally chromosomes such as *fig. 2*, *c*, *d*, *e* are of frequent occurrence. In this form of chromosome, the segments, which are more or less twisted upon themselves, are usually fastened to the spindle fibers near one end or at the middle. Such chromosomes are, as a rule, oriented tangentially upon the spindle.

As regards the development of the spindle, I have nothing

to add to my account of this process already published (Mottier, '97, '98). There are no individualized centrospheres or centrosomes. It may be mentioned further that in the mature spindle the following arrangement of fibers is to be observed: bundles of fibers extend from the chromosomes to the poles, a complex of fibers running uninterruptedly from pole to pole, the central spindle fibers, and those which diverge from the poles toward the plasma-membrane (*figs. 4, 5, 7*).

During metakinesis the daughter segments undergo a longitudinal fission which is at right angles to the plane of the first longitudinal splitting. In favorable cases, the result of this second longitudinal fission may be seen just as the daughter segments begin to separate or a little earlier, but as a rule the double nature of the daughter chromosomes is not evident until they have separated somewhat (*fig. 5*). As the daughter chromosomes separate, the granddaughter segments remain attached at the polar ends while the opposite ends generally diverge, and the well-known V-shaped structure is the result. In *fig. 5* it is clear that the V-shaped daughter chromosomes owe this form to the manner in which they are attached to the spindle and to the longitudinal fission of each daughter segment into granddaughter chromosomes. The chromosomes at this stage are confusing, because of the distortion of the segments due to the former twisting, and because of the fact that different forms of chromosomes with their various modes of attachment to the spindle occur in the same karyokinetic figure. It should never be forgotten, however, that under the circumstances just described in *fig. 5* the daughter chromosomes as they pass toward the poles are not always V's, but frequently straight or nearly straight rods which lie parallel and closely applied to each other (*fig. 8*, the chromosome in the middle). If the chromosomes be fastened to the spindle by the ends, the daughter chromosomes will form V's only when the equatorial ends of the granddaughter segments diverge.

In such chromosomes as *fig. 2, c. d, e*, it is apparent that the retreating daughter chromosomes may be either in the form of a U or V, and that this form will arise as a bending of the seg-

ments during metakinesis, since they are not attached by the ends. But such U's or V's are always double, as is shown in *fig. 6* on the left, and in *fig. 8*. Both limbs of the U or V are not necessarily of the same length, for there is every gradation between these forms and straight rods with slightly hooked ends.

The double character of the U-shaped daughter chromosome is not always very evident. Sometimes the granddaughter segments give the impression that one chromosome is lying just beneath another. Such phenomena were frequently observed by myself during my first investigation of the subject and were interpreted as two different daughter chromosomes, one lying directly below the other. Chromosomes which have the form of *fig. 2, c, d, e*, will probably yield U- or V-shaped elements as a result of being bent during metakinesis. The X and Y forms (*fig. 2, g, h*) will probably give rise also to V-, rod-, or hook-shaped elements. In *fig. 6*, the chromosome on the left was attached to the spindle near the middle, and each daughter segment consists of two similar U- or hook-shaped granddaughter elements. This chromosome may have been originally in the form of *fig. 2, b, e*, or the two daughter segments may have sustained the same relation to each other as in the figure, being in contact only at one end. In the chromosome at the right (*fig. 6*), the attachment was at the end, so that each daughter chromosome consists of two similar rods more or less bent or undulating.

Fig. 7 shows a typical nuclear figure in the anaphase. Here it is clear that the chromosomes were attached to the spindle by the ends, or very near the ends, so that as a consequence of the second longitudinal fission each daughter chromosome consists of two rod-like, straight, or variously bent or hooked granddaughter elements, which adhere at the polar ends, while the equatorial ends diverge. Sometimes the polar ends are so closely adherent or fused that the daughter chromosomes may appear as continuous V-shaped objects. In such cases it is often impossible to determine in what manner the V-shaped chromosome was produced, and it is this fact that has led to incorrect interpretations. The close adherence or fusion of the grand-

daughter segments at the ends turned toward the poles may be due to the manner in which the segments are attached to the spindle, as both granddaughter segments are fastened to the same bundle of fibers.

As the pairs of granddaughter elements arrive at the poles, they approach each other so closely that generally the identity of the individual elements can not be recognized, but in many cases the reconstruction of the daughter spirem can be followed with precision. I have already shown how the free ends of the daughter chromosomes unite to form a single daughter spirem (*fig. 10*). If the granddaughter segments form V's, the ends at the angle fuse first, in case this has not already happened, and then follows the fusion of the free ends. But in those cases in which the granddaughter segments are U-shaped and closely applied to each other side by side, it is evident that they must first separate in order to unite end to end, for the spirem was always found to be single.

At first the spirem is contracted, consisting of short turns, but soon after the nuclear membrane is laid down, the spirem becomes hollow and more regular and uniform.

Grégoire ('99) states with emphasis that in *Lilium candidum* and *L. speciosum* especially the chromosomes maintain their identity throughout in the daughter nucleus; that a continuous spirem is not formed but only a pseudo-spirem. My studies have included *L. candidum* and *Fritillaria persica*, and in these, as well as in *L. Martagon*, nothing is clearer than the fact that the chromosomes do lose their identity in the daughter nucleus.

The chromatin thread is not at all times an even and uniform spirem. During the reconstruction of the daughter nucleus, the chromosomes show a tendency to reticulate, becoming somewhat lumpy, and the spirem, if we may so designate the chromatin at this stage, is interrupted in many places. This is true especially just after the spirem begins to become more hollow, and the phenomenon may be regarded as a tendency to pass into the structure of the resting nucleus. The tendency of the nucleus to go into the resting stage is more pronounced in the embryo-sac and in other plants to be mentioned in a later paragraph.

In some cases and at a later stage a large part of the daughter spirem is quite regular, presenting a series of long and more or less uniform turns, so that when seen from the pole, the spirem appears in the form of a wreath or crown (*fig. 11*). This regularity is rather the exception than the rule, since in the majority of cases much of the spirem consists of irregular turns varying in size. It seems probable that were all the granddaughter elements rods, which form V's, the spirem would be quite regular; but, as often happens, when these elements arrive at the poles in the form of V's, double U's, and contorted rods intermingled, a very irregular spirem must result.

Strasburger, Guignard, and Grégoire state without reserve that the loops of the spirem (*fig. 11*) represent the V's and U's of the previous karyokinesis. This may be true, but the statement for Lilium, and more especially for other plants to be mentioned later, is purely a deduction. The spirem is often, if not always, continuous, as Strasburger also asserts, and the identity of the individual chromosomes is lost. It is true that in Lilium the daughter nucleus does not pass into that structure known as the complete resting stage, yet during the construction of the daughter spirem the chromosomes do to some extent become reticulated, so that the second mitosis may have for its purpose something more than the equal distribution of the granddaughter chromosomes to the four granddaughter nuclei. This seems especially true in those plants in which the daughter nuclei pass into the resting stage or approach it more closely than in Lilium.

The division of the daughter nucleus in the pollen mother-cell of Lilium and in other phanerogams as well, has been a stumbling block to every cytologist who has studied the problem. So complex is the process, especially the behavior of the chromatin, and so numerous are the difficulties attendant upon its study, that it is little wonder that errors have been made and that several different explanations have seemed at the time equally correct.

As I have shown in former publications, the spindle of the second mitosis develops as a multipolar structure, arising in a

manner similar to that of the first division. Attention was called to the fact also that the spindle fibers may enter the nuclear cavity before the spirem is completely segmented into the chromosomes, this being the case in both the pollen and embryo-sac mother-cells, and my present studies have only confirmed this statement.

Grégoire claims that in *Lilium candidum* and *L. speciosum* the chromosomes become isolated and independent before the nuclear membrane has disappeared. In some cases in *Lilium candidum* and *Fritillaria persica* it seems that the chromosomes are isolated before the nuclear membrane disappears, but this is rarely the case in *L. Martagon.* Moreover, no special importance has been attributed to the fact that the spindle fibers enter the nucleus in some cases before the individual chromosomes are completely isolated. It is not necessary, however, to demonstrate the identity of the chromosomes throughout the second mitosis to show conclusively that two longitudinal divisions of the chromatin occur during the first mitosis.

After the nuclear membrane has disappeared and the multipolar spindle complex is laid down, or in the process of formation, the chromosomes are, as a rule, so closely entangled, kinked, and twisted that it is impossible to trace them save in exceptional cases. For this reason it is extremely difficult, if not impossible, to determine beyond all doubt the manner in which they are formed from the spirem. *Fig. 12* represents a section of a daughter nucleus in the multipolar stage of the spindle. In this case the spirem is more loosely arranged, and many of the loops and turns may be traced with greater accuracy than in the vast majority of instances. From this and *figs. 13, 14,* and *15,* it is evident that the two segments of each chromosome in the nuclear plate represent probably the two parallel limbs of the loops observed at an earlier stage, which have become more or less closely applied to and sometimes twisted upon each other. The fact that the turns of the spirem in the daughter nucleus are not all regular and uniform accounts for the twisted and entangled condition of the chromosomes during the formation of the spindle. In the mature spindle (*figs. 14, 15*) the chromo-

somes are arranged both radially and tangentially. The two elements of each chromosome may lie parallel with each other, or they may remain almost entirely separate, being in contact only at the ends that are attached to the spindle fibers (*fig. 14*). This seems to be more frequently the case in *Lilium candidum* and Fritillaria. In the latter arrangement both elements may lie upon the same side or upon opposite sides of the equatorial plane. The chromosomes are fastened to the spindle fibers at or near the ends, rarely near the middle. When they are fastened near the ends, these are generally bent somewhat so that the retreating segments appear in the form of rods which are slightly hooked at the ends turned toward the poles (*fig. 17*).

Strasburger (1900), Guignard, ('99), and Grégoire ('99) claim that the two segments of each chromosome in the second division correspond exactly to the pairs of granddaughter segments which form the V's or U's of the first mitosis, and that they are fastened to the spindle at the places of bending or at the angles of the V's as in the first mitosis. Theoretically there may be little objection to this statement, as the matter in question may be a detail of secondary importance, but it seems that the observed facts will not admit of its general application in Lilium. *Figs. 13, 14,* and *15* show clearly that the free ends of the loops or V's are as likely to be attached to the spindle. Furthermore, we cannot say with certainty that the chromosomes of the second mitosis are identical with the pairs of granddaughter elements of the first division, for it does not seem impossible that the spirem may segment in such a manner that the segments of each chromosome of the second division may be derived from different pairs of granddaughter elements.

This may be brought about by the spirem segmenting at the apex of the V's rather than at the points corresponding to the free ends of the elements of each V, assuming that each long loop of the spirem represents a V. Under such circumstances, if all the granddaughter elements composing the spirem were so oriented in the preceding anaphase as to form V's, then all of the chromosomes of the second division would be made up of elements from different pairs of sister segments. But on the

other hand, should the spirem segment in some places at the points corresponding to the angles of the V's, and at others which represent the points of contact of the free ends of the V-shaped chromosome, then some of the chromosomes of the second division would be composed of sister segments while others would not. If either of the last named possibilities be true, it might very easily happen that sister segments would go to the same granddaughter nucleus, a condition that might furnish a probable basis for a greater variation. Even though the latter supposition be true — that some or all of the chromosomes are not sister segments—it is possible that the results would be the same, for the spindle, or whatever part of the cell that has to do with the distribution of the chromosomes, may be able to prevent any such mixing by distributing to the granddaughter nuclei those chromatin elements which would unavoidably go to each if every chromosome were identical with the pairs of sister segments of the preceding division.

But since the chromosomes lose their identity in the daughter nucleus, neither of the foregoing suppositions can be objectively demonstrated. This is not possible, as yet, even in those cases in which the spirem is very regular or in plants with fewer chromosomes, and the possibility is still farther removed in those cases of more frequent occurrence in which the spirem is very irregular.

In the foregoing statements the writer does not intend to convey the idea that he rejects the older view, but the doctrine is advanced largely as a probability which seems to have some basis in fact. The older view, that the chromosomes of the nuclear plate of the second mitosis are identical with the pairs of granddaughter segments of the first division, is the simpler hypothesis, involving fewer complications, and for that reason it may be more readily accepted. Its truth, however, can be no more definitely demonstrated than that which I have suggested as a possibility, for there is no known angiosperm, so far as the writer is aware, in which the chromosomes of the first division do not lose their identity in the daughter nucleus, and we do not know with absolute certainty whether the daughter spirem seg-

ments in such a manner that the resulting pairs of segments are identical with the pairs of granddaughter segments of the first division. In those cases to be mentioned in a later paragraph, in which the daughter nucleus closely approaches or passes into the resting stage, it is readily seen how much more difficult the problem is.

A glance at those stages of the process represented in *figs. 12, 13, 14,* and *15* will give some idea of how the long and entangled chromatin segments are gradually brought to a more regular arrangement in the nuclear plate, although in species of Lilium with long chromosomes the arrangement, as a rule, is not very regular. The twisted and contorted form of the chromosomes seems to be due chiefly to the irregularity of the spirem.

During the transformation of the multipolar into the bipolar spindle, the entangled collection of chromosomes seems to be gradually pulled, pushed, and untangled into the more regular arrangement of the equatorial region, and there can be little doubt that the spindle fibers are primarily active in bringing each pair of chromosomes into its proper place.

As already stated, the segments of the chromosomes in the nuclear plate lie generally side by side and more or less twisted about each other. Sometimes the free ends of the segments diverge widely, those which are fastened to the spindle fibers being in contact or even separated a little. This phenomenon has been interpreted by some observers as metakinesis, but such a condition is sometimes met with before the spindle is mature. The spindle fibers have an orientation similar to that of the first mitosis.

As the segments separate during metakinesis, the nuclear plate presents a complicated picture (*fig. 16*). This phenomenon is due to the twisted condition of the chromosomes previously mentioned. On their way to the poles the segments may straighten out somewhat. Each is then usually a long and frequently undulating or bent rod, that is generally hooked at the end to which the spindle fibers are attached (*figs, 17, 18*). Sometimes the chromosomes are attached near the middle, in which case the retreating segments will be in the form of a V or a U.

The formation of the granddaughter nuclei presents nothing which demands a special mention.

Confining himself to the study of the chromosomes in Lilium alone, the investigator would undoubtedly hesitate long before concluding in favor of the double longitudinal splitting of the chromosomes during the first mitosis, because of the variety existing in the form of the chromosomes and their orientation upon the spindle, the difficulty with which the double nature of the V-shaped elements which owe their form to a bending is generally recognized, and because of the extremely complicated character of the chromatin spirem during certain important stages of the second mitosis. The phenomena presented in *Podophyllum peltatum* and *Tradescantia virginica* leave no doubt whatever as to the correctness of this view.

The development of the chromosomes during the prophase of the first division in Podophyllum, as well as other important detail of karyokinesis, have already been fully described in my earlier paper (Mottier, '97), so that only enough of the process will be given here to bring out clearly the behavior of the chromosomes.

Soon after the segmentation of the spirem into chromosomes the daughter segments tend to become separated except at one or both ends, or they may adhere only near the middle and both ends diverge, as the daughter segments are usually somewhat curved. They may also remain in contact for their entire length, but this is less frequently the case. When the chromosomes are arranged in the nuclear plate they lie, as a rule, tangentially upon the spindle. The daughter segments are more frequently observed adhering at one end only as in *fig. 20*, but, as in Lilium, should the segments adhere at both ends, the ring-shaped chromosome is the result (*fig. 19*). It not infrequently happens that both forms of chromosomes are seen in the same nuclear figure. *Figs. 19* and *20* show the manner in which these chromosomes are fastened to the spindle fibers. In those cases in which the daughter segments adhere only at one end, the ends in contact are so closely applied as to give the appearance of a lump or swollen place. This is especially true when the ends of the curved segments are turned directly toward or away from the

observer. In case the daughter segments are in contact for their entire length the chromosomes may be fastened to the spindle at one end and arranged radially upon it; but, as a rule, the point of attachment is at the place of bending, whether that be near one end or at the middle of each segment. The X-, Y-, and U-shaped chromosomes, although of less frequent occurrence in Podophyllum, are formed in the same manner as described for Lilium.

During the metaphase, or immediately following it, each daughter chromosome is seen to be double or composed of two similar granddaughter segments, the result of the second longitudinal splitting (*figs. 22, 23*). The shape of the pairs of granddaughter segments will, of course, depend upon that of the daughter chromosome. In *fig. 21, a*, it is evident that this chromosome before metakinesis was similar to those in *fig. 20*, In *fig. 21, b*, the daughter segments may have been in contact throughout and the chromosomes fastened to the spindle at the end. The retreating pairs of segments in this case may present the familiar V's. The chromosome to the right in *fig. 22* was either in the form of a ring or a U when in the nuclear plate. This condition was observed at the time of my earlier study upon Podophyllum, but such conditions were interpreted as two similar but different chromosomes lying close together, one beneath the other.

The double nature of the daughter chromosomes can be clearly distinguished until they reach the poles. Having arrived at the poles, they generally become closely crowded together so that the individuals are difficult to distinguish. It can be seen, however, that the segments of each pair tend to separate, and there was nothing observed which might lead one to conclude with any certainty that a double spirem is formed. The granddaughter segments in forming the spirem tend to reticulate somewhat so that they become irregular in shape and their exact identity is lost. Those observers who assert without reserve that the identity of the chromosomes can be followed at all times from one division to the other, certainly leave this fact and other steps of the process out of consideration.

In a short time, a more regular spirem is formed in which parallel or adjacent portions are frequently connected by fine threads. As I have already shown, the spindle of the second division arises as a multipolar structure (*fig. 26*). During the formation of the spindle, the chromatin spirem segments into the chromosomes. The segments are collected together within the complex of spindle fibers so that the manner of segmentation can not be determined with certainty. The same would be true if the spirem segments before the nuclear membrane had disappeared, and it is a matter of little importance whether the spirem segments before or after the disappearance of the nuclear membrane. In many cases it seems that the spirem breaks up into pieces equal in length to the two segments of the chromosomes, but this does not always appear to be true (*fig. 26*). However, during the transformation of the multipolar complex of fibers into the bipolar spindle, the chromosomes are gradually arranged more regularly into the nuclear plate (*figs. 27, 28*). Each chromosome consists now of two similar segments which may or may not be closely applied to each other. Instances have been observed in which the two segments of a chromosome were of a slightly different length. The chromosomes are probably identical with the pairs of granddaughter segments of the first division, but here, as in Lilium, we do not know definitely whether the two segments of each chromosome are sisters. The chromosomes are mostly in the form of rather thick rods more or less curved. They are fastened to the spindle fibers near the ends or at the place of bending. The retreating segments, therefore, may be nearly straight, hooked, V- or U-shaped.

Among monocotyledonous plants, apart from the lilies, few perhaps are more suitable to demonstrate the fact of a double longitudinal splitting of the chromosomes during the first mitosis in the pollen mother-cells than *Tradescantia virginica.*

Between the time of the segmentation of the chromatin thread into chromosomes and the development of the spindle, the chromosomes contract greatly, becoming shorter and thicker. The daughter segments tend to separate except at one or both ends. In case they adhere at both ends, a ring or ring-like chromo-

some results. At the time of or even before the formation of the spindle, the daughter segments contract to such an extent that each may be in the form of a short, thick crescent. Each ring-like chromosome will therefore consist of two crescents whose ends adhere (*fig. 30*). It not infrequently happens that the daughter segments contract in such a manner that closed rings are formed. If the daughter segments remain adhering at one end, each chromosome in the nuclear plate is seen to consist of two thick pieces in the form of either a short crescent, a broad U, or only slightly bent at one or both ends (*fig. 31*, on the left). Under these circumstances the whole chromosome is relatively long, reaching almost from pole to pole.

As a rule the chromosomes are oriented tangentially upon the spindle, the fibers being fastened at the middle of each segment or near one end. It is interesting to note that the place to which the fibers are attached is almost always in the form of a small protuberance, giving the impression that a pull is exerted by the spindle fibers. In *fig. 30* is shown the singular phenomenon of two chromosomes fastened together, a fact which is of course without any special significance.

No indication of a second longitudinal splitting is seen in Tradescantia when the chromosomes are in the nuclear plate, and, so far as could be determined with certainty, not even during metakinesis, although it may occur here as early as in Lilium (*figs. 30, 32*); but by the time the daughter chromosomes have traversed one-half the distance toward the poles, it can be usually seen that each consists of a pair of granddaughter segments, arising from the second longitudinal splitting (*fig. 33*).

Having arrived at the poles, the pairs of granddaughter segments become somewhat crowded together, and shortly the nuclear membrane is laid down (*fig. 35*). The segments, or some of them at least, now separate slightly; but as they begin to reticulate, becoming lumpy and very irregular, their identity is soon lost. The process of reticulation continues, and at a little later stage the lumpy chromatin masses which are connected by numerous fine threads present the appearance of *fig. 36*. As in many other plants, there is not an even and uniform

spirem formed at once from the segments of the daughter chromosomes. The reticulation continues, at least in many cases observed, until a structure is reached that is certainly very near that of a resting nucleus. *Fig. 37* represents a nucleus seen from the pole. If the same nucleus were observed from the side, at this or even a little earlier stage, its chromatin would present the appearance of a rather thick and irregular spirem.

There are phenomena in Tradescantia which would suggest that the daughter spirem or parts of it are formed double, but all the evidence taken together seems to indicate that only a single spirem is the result. Daughter nuclei were observed in which certain parts of the spirem, or chromatin thread, seemed to be double, while other parts showed nothing of a double character. The chromatin of the daughter nucleus does not form the regular and uniform spirem to be observed in other monocotyledonous species and in Podophyllum, and for this reason the differentiation of the chromosomes is followed with greater uncertainty. With the approach of the second mitosis, the chromosomes are differentiated and lie distributed in the nuclear cavity before the disappearance of the nuclear membrane. They are often very irregular in shape and connected by delicate linin threads. Even when arranged in the nuclear plate, they present an irregular, lumpy, and twisted form. In those cases in which the chromosomes are most regular, each is seen to consist of a pair of rod-shaped segments which are either straight with only a slight bend at one end, or more or less undulating and twisted (*fig. 39*). As in Lilium, some of the chromosomes are oriented radially and some tangentially upon the spindle.

It may be that the two segments composing each chromosome are sisters, and that the chromosomes are identical with the pairs of granddaughter segments of the first division, but the possibility of an objective demonstration of this supposition seems out of the question.

The process of the second karyokinesis in the pollen mother-cells of other plants seems to suggest strongly that the division of the daughter nuclei has some other purpose than merely the

distribution of an equal number of chromosomes to the four granddaughter nuclei.

In 1898, the writer showed clearly the striking similarity between the first two mitoses in the micro- and macrospore mother-cells of *Helleborus foetidus* and *Lilium Martagon*, and concluded upon that evidence that the pollen and embryo-sac mother-cells were homologous. The work of recent observers, especially Juel (1900) and Schniewind-Thies (1901), who have confined their attention primarily to this phase of the problem, has only confirmed this view.

Since the chromosomes in the pollen mother-cell undergo a double longitudinal division during the first or heterotypic mitosis, the same would seem certain also in the embryo-sac mother-cell, and it was for this reason that a reinvestigation of the problem in *Lilium Martagon* was undertaken.

As was expected, the first and second mitoses in the embryo-sac were found to be essentially like those in the pollen mother-cell. These divisions will be described somewhat in detail in order to show what the embryo-sac contributes toward the solution of this and other problems suggested in the preceding pages.

As is well known, the mother-cell of the embryo-sac of Lilium becomes the macrospore and develops at once into the embryo-sac. The first nuclear division is heterotypic, the nucleus undergoing the same changes during the prophase as in the pollen mother-cell. The spindle arises as a multipolar structure, and the form and orientation of the chromosomes within the multipolar complex of fibers and upon the mature spindle present nothing requiring a special description.

Owing to the small number of macrospores in an ovary as compared with the number of microspore mother-cells in an anther, a much smaller number of karyokinetic figures will be observed in the former, yet in the course of an extended investigation, it seems exceedingly probable that about the same variety in the form of the chromosomes exists in the macro- as in the microspore mother-cell. As a rule, the daughter segments are twisted upon each other and fastened to the spindle fibers by the ends (*fig. 40*). Only in exceptional cases can any indication of

the second longitudinal splitting be discerned before metakinesis. As soon as the daughter chromosomes separate, however, each is seen to consist of two similar granddaughter segments. The figures which these pairs of segments present will depend, of course, upon the orientation of the daughter chromosomes toward each other, their shape, and mode of attachment to the spindle fibers.

The retreating segments, therefore, may form V's, U's, or more or less contiguous rods, which may be undulating or abruptly bent, and, as is sometimes the case, hooked at the ends directed towards the poles (*fig. 41*).

Having reached the poles, the granddaughter segments do not, at least as far as my observations have extended, form immediately a regular spirem of a uniform thickness, but there is here a greater tendency on the part of the chromatin elements to become reticulated and pass into a structure closely approaching that of a resting nucleus. This was found to be true in all cases observed. As will be seen from *fig. 42*, the two daughter nuclei present a structure which is certainly very near that of a resting nucleus. Several nucleoli are present, and there is no regular, continuous spirem. Although the nuclear thread may be continuous, yet the chromatin is in the form of granules that are of a very variable size and unevenly distributed. Certain portions of the nuclear thread contain apparently a single row of small chromatin granules, as in an early prophase of karyokinesis; in other portions two rows of these granules are present, while still other parts are thicker and somewhat lumpy.

In the light of all the facts observed, nothing seems more certain than that in the daughter nuclei all identity of the chromosomes is lost from observation. While it is true that a greater pause intervenes or seems to exist between succeeding nuclear divisions than between the first and second mitoses, yet it does not follow that the second division takes place immediately after the first without a resting pause.

As regards the details of further karyokinetic activity in the embryo-sac, I am able to add little to my former observations. As already mentioned (Mottier, '98), the second mitosis bears

certain striking resemblances to that in the pollen mother-cell. In all probability they are exactly the same. In the chromatin spirem no indication of a longitudinal splitting is to be seen. The nuclear membrane disappears as such, and the spindle fibers penetrate the nuclear cavity before the spirem is segmented into chromosomes (*fig. 43*). The complete segmentation seems to be effected later than in the pollen mother-cell. Within the multipolar complex of spindle fibers the partly segmented spirem, as in the pollen mother-cell, is generally greatly entangled and kinked. As the spindle matures, the arrangement of the chromosomes becomes more regular, but, as a rule, less so than in *fig. 44*. When the spindle has reached the typical bipolar stage, the chromosomes are oriented in the nuclear plate, some radially and some tangentially (*fig. 44*).

Each consists of two rather long and closely applied segments that may be twisted upon each other. It seems that the spirem may not be entirely segmented even when the typical bipolar stage of the spindle is reached (*fig. 44*), and as the apparently unsegmented portions are double nothing seems more reasonable than the conclusion that the double nature is due to the longitudinal splitting of the chromatin spirem, and it was upon this evidence that my conclusion of 1898 was based, namely, that the spirem of the daughter nucleus undergoes a longitudinal fission.

Since the second longitudinal fission takes place during the first mitosis, it is not difficult to understand, especially when one bears in mind the entangled or kinked condition of much of the spirem during the development of the spindle, and the fact that portions of it are very slender, appearing as if stretched by the fibers, how parallel portions of the spirem may be so brought in contact as to look like a longitudinal splitting had occurred. Further evidence in favor of the doctrine that the two segments of each chromosome are brought in contact side by side, is the fact that in both pollen and embryo-sac mother-cells one very frequently meets with chromosomes that do not consist of two rather straight and readily distinguishable segments, but rather short, thick, and lumpy chromosomes which show that the segments are kinked or twisted up into a short spiral or coil. It

seems furthermore exceedingly probable that the longer and more uniform turns of the spirem will furnish the regular and straight chromosomes, whilst the less regular parts—for such exist in the majority of the daughter nuclei in question—give rise to the short, kinked, or knotted ones.

It may be mentioned in passing that every step in the development of the spindle seems to bear direct evidence in favor of the view that the arrangement of the chromosomes is accomplished by a pushing and pulling action of the spindle fibers.

Relative to the behavior of the chromatin, it may be stated finally that the possibility is not excluded that in some cases the spirem may be formed double, in part or entirely, but direct evidence of such a possibility was not observed save in the presence of two rows of chromatin granules in portions of the thread-work of the daughter nucleus.

The daughter nucleus in the lower end of the embryo-sac, which gives rise ultimately to the antipodal cells and the lower polar nucleus, behaves in division exactly as the upper, and the fact that this nucleus presents a larger number of chromosomes only adds more difficulties to the problem of the chromosomes.

In certain respects, the third karyokinesis in the embryo-sac of *Lilium Martagon* resembles the second. This is true as regards the development of the spindle whose fibers penetrate the nuclear cavity before the chromatin spirem is entirely segmented into the chromosomes (see Mottier, '98, *figs. 16, 17*). The behavior of the chromatin, however, is quite like that in ordinary and typical vegetative cells.

The first two mitoses in the spore mother-cells of higher plants, therefore, are heterotypic and homotypic respectively, these terms being used in the sense of Flemming. The chief distinguishing characteristic of the heterotypic nuclear division is the double longitudinal fission of the chromosomes. The shape of the chromosomes is without importance, since the form of the individual chromatin elements, which often varies in the same nucleus, is due to different causes. This division is further characterized in plants by a rather long period of growth during the prophase which results in a marked increase in the size of

the nucleus and in the quantity of chromatin substance. In the higher plants, it is during this division that the reduced number of chromosomes appears.

In the homotypic division, the chromatin spirem or thread does not undergo a longitudinal fission. During the development of the spindle the granddaughter chromosomes are arranged in pairs in the nuclear plate by means of the spindle fibers. The segments of each pair often lie closely applied side by side in such a way as to suggest that the daughter spirem had split longitudinally. This arrangement of the segments with reference to each other does not always obtain, for they may frequently diverge, one lying tangentially upon one side of the equator and the other upon the other side. In the pollen mother-cell of Lilium and in certain other plants, the homotypic may follow the heterotypic division without the intervention of a complete resting stage, but even here there is a greater or less tendency for the daughter nucleus to pass into the structure of the resting condition. In all cases, however, the identity of the individual chromosomes is lost from observation in the daughter nucleus.

THE HOMOLOGY OF THE MICRO- AND MACROSPORE MOTHER-CELLS.

From the foregoing statements and in the light of the recent researches of Juel (1900) and Schniewind-Thies (1900), it seems that all cytological evidence indicates clearly and unmistakably that the pollen mother-cell and embryo-sac mother-cell are homologous.

The writer has already shown that the first nuclear division in the macrospore mother-cell of Helleborus is heterotypic, and the same is true for Podophyllum. In the development of the embryo-sac of Helleborus, which may be regarded as typical for dicotyledonous plants, as well as for many monocotyledonous species, the heterotypic and homotypic mitoses are each followed by cell-divisions, giving rise to four potential macrospores, homologous with the four granddaughter cells in the pollen mother-cell. The fact that occasionally in Helleborus cell-division does not take place until after the second mitosis

serves to show more definitely the close resemblance between embryo-sac and pollen mother-cell (Mottier, '98, *fig. 31*).

Juel (1900) has shown that the two successive divisions in the macrospore mother-cell of Larix gives rise to four cells in the same manner as in such angiosperms as Helleborus, and that the first of these mitoses is heterotypic. This is probably true for many other gymnosperms.

It is well known that the developmental history of the embryo-sac between the macrospore mother-cell and the mature female gametophyte is shortened in certain cases by one or two nuclear divisions, and it is important to know, in these cases of abbreviated development, the place occupied by the two mitoses in question.

With this object in view Frau Schniewind-Thies (1900) has investigated the development of the embryo-sac in several species of the Liliaceae, and has brought together a number of interesting observations. In all cases examined by this author, the first two nuclear divisions in the mother-cell are heterotypic and homotypic respectively, and whenever four potential spores result, each nuclear division is followed by a cell-division. One of these potential macrospores, usually the lower one, develops, as in Helleborus, by three successive typical or ordinary mitoses into the embryo-sac. In the case of *Scilla sibirica*, however, the developmental history of the female gametophyte, or embryo-sac, is shortened by only one nuclear division. The first or heterotypic division is followed by a cell-division. Following the second, or homotypic mitosis, no cell-division takes place, and consequently each daughter cell contains two nuclei. Either of these cells may function as the macrospore, and develop into the embryo-sac, while the other is absorbed. In this case the homotypic division represents the first mitosis in the spore. The two daughter nuclei now divide twice in the typical way to give rise to the mature embryo-sac. In *Scilla sibirica*, therefore, there is a shortening of the development by one typical mitosis.

In Lilium and in other genera with a similar development of the embryo-sac, the process is further shortened. Here the macrospore mother-cell functions at once as the spore. No cell

division follows the heterotypic or homotypic mitoses, although there is sometimes a tendency toward cell-plate formation (Mottier, '98), and these two nuclear divisions represent two out of the three divisions in the embryo-sac.

In the light of these facts, it seems clear that in Lilium there is merely a shortening of the development of the embryo-sac by two typical mitoses.

The development of the embryo-sac immediately from the mother-cell has probably no parallel in the case of the pollen, since Strasburger (1901) and Frye (1901) have recently shown that in Asclepias and in a few other genera, in which the pollen mother-cell was supposed to function as the spore, the usual process of the development of the pollen obtains.

SUMMARY.

In the karyokinetic processes occuring in the micro- and macrospore mother-cells of the angiosperms, certain phenomena may now be regarded as very well understood and pretty definitely established.

In the first place, the nuclear spindle arises as a multipolar complex of fibers, wholly or almost wholly of cytoplasmic origin, which is gradually transformed into the typical bipolar spindle. No centrospheres or centrosomes are present.

During the first mitosis the reduced number of chromosomes appears. There is no reduction or qualitative division in the sense of Weismann. The first division is characterized further by two longitudinal divisions of the chromatin, the second longitudinal splitting being at right angles to the plane of the first.

The first longitudinal fission occurs in the early prophase; the second is demonstrable in the mature spindle when the chromosomes are arranged in the nuclear plate, or first during the meta- or anaphase. Sometimes evidence of the second splitting appears at an earlier stage, but these are not conclusive.

The second longitudinal fission seems to be a preparation for the second or following mitosis.

The first division is also characterized by the long period of

growth during the prophase, resulting in an increase in the size of the nucleus and in the quantity of chromatin.

The granddaughter chromosomes, having arrived at the poles, unite end to end to form a continous daughter spirem in Lilium and in Podophyllum. In all species examined all identity of the individual chromosomes is lost in the daughter nucleus. In Podophyllum and even in Lilium the granddaughter chromosomes show a marked tendency to reticulate, becoming irregular, while in Tradescantia and in the embryo-sac of Lilium the reticulation of the chromatin is continued to such an extent that the daughter nucleus passes almost into the structure of the resting stage.

The chromatin thread or spirem of the daughter nucleus does not undergo a longitudinal fission. During the development of the spindle and in some cases earlier, the daughter spirem segments into the granddaughter chromosomes, which are gradually arranged in pairs in the nuclear plate by means of the spindle fibers. As the granddaughter chromosomes are conveyed to the poles during the anaphase, they are usually in the form of rods, which may be straight, undulating, hooked at one end, or sometimes U- or V-shaped.

The first mitosis in both the micro- and macrospore mother-cells of the higher plants is, therefore, heterotypic, and the second homotypic. These nuclear divisions are not, properly speaking, reducing or reduction divisions. They are not the agents of the reduction, but rather the result of the numerical reduction of the chromosomes.

In the plants which were the objects of investigation, it is clear that the various forms of chromosomes observed in the heterotypic mitosis are such that might suggest more than one valid interpretation, and it is in a large measure due to this fact that there still prevail different and opposing interpretations in plants as well as in animals. The history of the development of the chromosomes, and a more thorough knowledge of their behavior during every phase of karyokinesis have shown that the shape which they may assume is of secondary importance. The form of each chromosome depends chiefly upon the shape of the

daughter segments and their orientation toward each other and the manner in which the chromosome is fastened to the spindle.

In Lilium the daughter segments are, as a rule, rather long rods closely applied to and twisted upon each other. As shown in the preceding pages, there are many variations from the usual form. They are generally fastened to the spindle fibers at the end, and under such circumstances placed radially upon the spindle.

During metakinesis, the second longitudinal fission manifests itself, and each daughter chromosome consists of two granddaughter segments, which usually adhere and sometimes fuse at the ends to which the spindle fibers are fastened, while the opposite ends may diverge to form the V- or U-shaped figures. It often happens that the free ends of the granddaughter segments do not diverge, in which case the retreating daughter chromosome is in the form of two contiguous or nearly contiguous rods. Sometimes the ends of the granddaughter segments appear so completely fused near the place of fastening to the spindle that the daughter chromosome resembles a continuous rod bent into the shape of a V or U. On the other hand, V- and U-shaped daughter chromosomes are brought about by another process, namely, that of a bending. Under this circumstance the chromosomes are attached to the spindle, not by the ends, but near the middle or at some distance from one end, and the orientation upon the spindle is generally tangential. The V- or U-form is then due either to a previous bending of each daughter segment in such a manner as to form a ring or loop, or the bending may be caused by a pushing or pulling of the spindle fibers during the meta- or anaphase. In all such cases the V's or U's so formed are double as a result of the second longitudinal splitting.

The fact that in many plants the daughter nucleus passes into the resting stage would seem to indicate that the second or homotypic mitosis signifies something more than the mere distribution of the granddaughter chromosomes to the granddaughter nuclei. In such cases it is difficult to understand the purpose of two longitudinal fissions during the first karyokinesis.

It is generally assumed that the two granddaughter segments of each chromosome appearing in the nuclear plate of the second mitosis are sisters and identical with the pairs of segments of the anaphase of the first division. This can only be true when the spirem segments in exactly the same manner in which it was constructed, assuming that the pieces separated by the breaking up of the spirem consist of the two segments of each chromosome. But on the other hand, if the spirem break up so that the individual segments are separated from the start, then sister segments may be brought together in pairs in the nuclear plate. It is evident in many instances that the pieces into which the spirem falls does consist of the two segments of each chromosome in the second division, and that these are brought side by side by the folding of the piece or by the two parallel members of a loop or turn approaching each other.

However, as has been pointed out in a preceding paragraph, should the spirem segment in a different manner with each piece representing two segments, the two segments of the chromosomes would not necessarily be sisters. In such cases, the possibility is not excluded that two or more sister chromosomes may fall to the same nucleus. Moreover, as the identity of the individual chromosomes is lost in the daughter nucleus, it is not possible to demonstrate objectively whether the segments are sisters or not.

There is also no basis in fact which will justify the doctrine that one chromosome is qualitatively different from another.

The development of the spindle seems to show that the chromosomes are oriented in the nuclear plate by a pushing and pulling action of the spindle fibers. There is some evidence to indicate also that during the anaphase a pushing is exerted upon the retreating segments, yet this view can not be accepted without the greatest reserve, as the presence of a large bundle of fibers extending from each daughter segment to the poles argues strongly in favor of a pulling or contracting of the fibers.

According to the best cytological evidence the micro- and macrospore mother-cells are homologous. That type of development of the embryo-sac in which four potential macrospores

are produced as the result of the heterotypic and homotypic mitoses, occurring in gymnosperms as well as in the majority of angiosperms, is regarded as the more primitive, while that typified by Lilium is interpreted as a derived condition.

INDIANA UNIVERSITY,
Bloomington, Ind.

*** In justice to the author it should be stated that the publication of the foregoing paper has been prevented for six months by unreasonable delay in the lithographing of the plates.—EDS.

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EXPLANATION OF PLATES XI–XIV.

All figures were drawn from sections with the aid of the Abbé camera lucida and with Zeiss apochromatic homogeneous immersion 2 mm., apert. 1.40, with compensating oculars 6 and 8, or with Leitz homogeneous immersion $\frac{1}{16}$ and oculars 1 and 3.

FIGS. 1–18. Pollen mother-cell of *Lilium Martagon*. *Figs. 1–11* refer to the first mitosis; *12–18* to the second.

FIG. 1. *a*, *b*, *c*, and *d* represent four chromosomes shortly after the cross segmentation of the spirem; in *a* the daughter segments are somewhat closely applied and twisted upon each other; in *b* the ends show a tendency to separate and diverge; and in *c* the segments are almost separate save at one end; *d* is a case in which the chromosome is somewhat bent and the segments are in close contact near the middle. × about 1750.

FIG. 2. Various forms of chromosomes observed in the nuclear plate: *a*, the chromosome is fastened to the spindle at the end, and consists of two rod-shaped segments twisted upon each; *b*, the two segments which adhere at both ends bend out at the middle to form the ring-shaped chromosome; such chromosomes are generally placed tangentially upon or within the spindle; *c* to *h* represent several other forms of chromosomes commonly met with; the bundle of fibers shows the method of attachment to the spindle; *f* is an X-shaped chromosome, although in this form of chromosome the ends may diverge more; *h* is a typical Y-shaped chromosome. *a* and *b*, × 1750; *c* to *h*, × 1500.

FIG. 3. A nearly mature spindle; the chromosomes are being arranged in the nuclear plate. × 1500.

FIG. 4. A mature spindle showing only three chromosomes. × 1500.

FIG. 5. Chromosomes in metakinesis; each daughter chromosome consists of two granddaughter segments as the result of the second longitudinal splitting; the granddaughter segments adhere at the ends to which the bundle of spindle fibers is fastened, while the opposite ends diverge to form the familiar V-shaped figures; the chromosomes in this figure are all fastened to the spindle by the ends. × 1500.

FIG. 6. Two chromosomes from a karyokinetic figure in the same stage as *fig. 5*; the chromosome at the right was fastened endwise to the spindle, and consequently the retreating pairs appear as two rods lying side by side or as V-like figures; the chromosome at the left was in the form of either a complete open or an incomplete ring: the daughter segments are therefore somewhat U- or V-shaped with one limb of the U, or V a little longer than the other; the V-shape here is due to a bending; each U or V is double as a result of the second longitudinal fission. × 1750.

FIG. 7. Median section of an entire pollen mother-cell. The chromosomes are in the anaphase; the granddaughter segments form V's or pairs of nearly parallel rods. × 1500.

FIG. 8. The same stage as the preceding; the daughter chromosomes on the right and left consist each of two U- or hook-shaped granddaughter segments owing this form to a bending; the granddaughter segments of those in the middle are contiguous rods slightly bent or hooked at one end. × 1500.

FIG. 9. Five daughter chromosomes nearly at the poles; the granddaughter segments which are at a slight distance apart are connected by fine threads. × 1750.

FIG. 10. The granddaughter segments are forming the spirem of the daughter nucleus by uniting end to end; between the daughter spirems extends the system of connecting fibers. × 1500.

FIG. 11. Daughter nucleus seen obliquely from the pole side; the spirem is arranged chiefly in long and regular turns. × 1750.

FIG. 12. A multipolar spindle of the second mitosis; the loops and turns of the partly segmented spirem are entangled; some of the free ends were made by the knife in sectioning; as a rule, at this stage the spirem is much more entangled and kinked, so that it is impossible to trace the individual pieces; the chromosomes are probably being pushed into the nuclear plate. × 1750.

FIG. 13. The spindle is bipolar, but the chromosomes are not completely oriented into the nuclear plate; it seems also that the spirem is not wholly segmented.

FIGS. 14, 15. The spindle is about mature, and the arrangement of the chromosomes is typical except that they are very often much more irregular, twisted, and kinked; in these two figures it is clearly seen that some of the segments of the chromosomes are twisted upon each other, while others are separate, lying tangentially (*fig. 14*) upon opposite sides of the equator. × 1750.

FIG. 16. Chromosomes in metakinesis; the separating elements are often variously bent or curved.

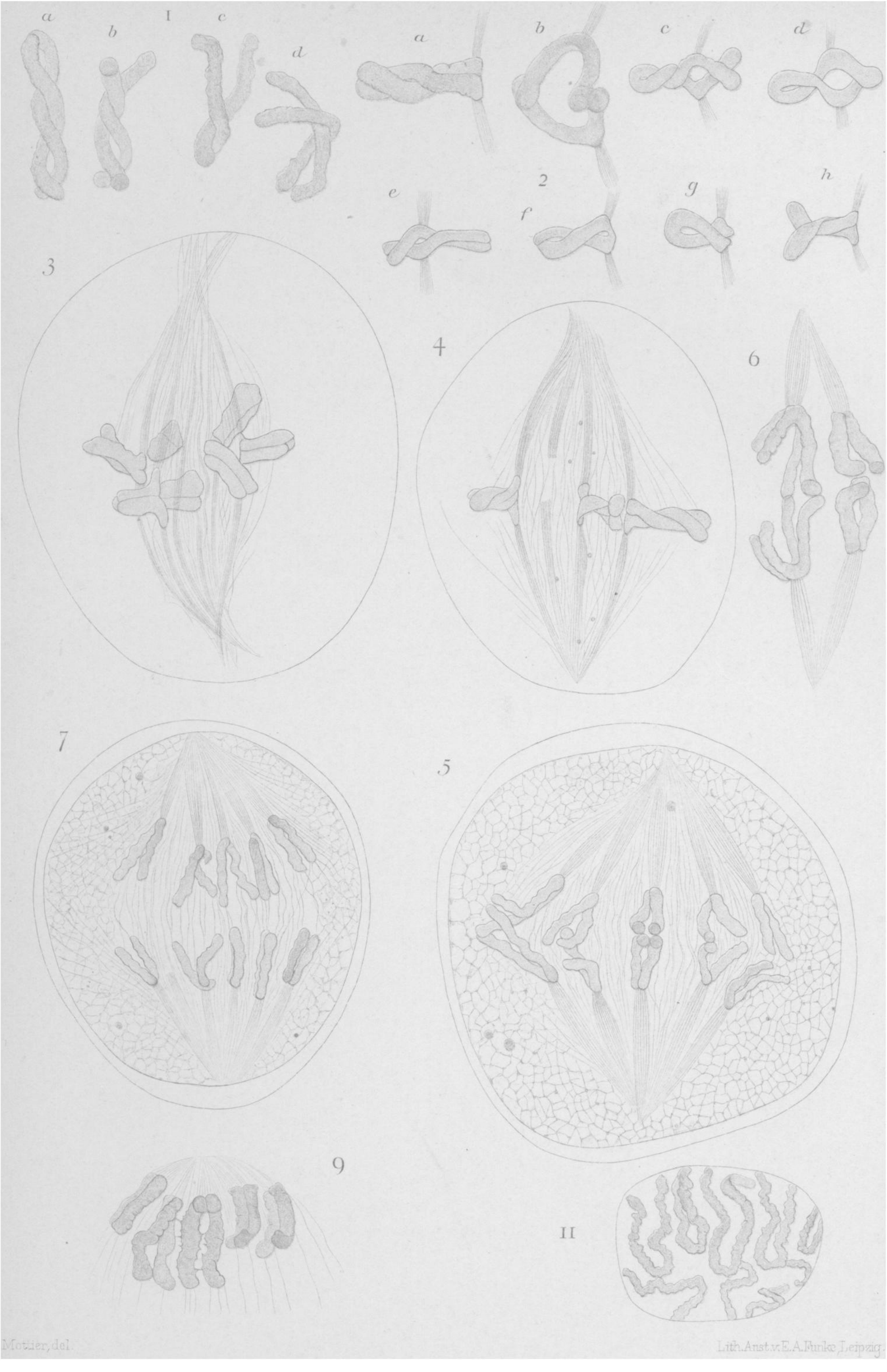
FIGS. 17, 18. Two spindles in anaphase; the retreating chromosomes are more or less curved or undulating rods which are almost invariably slightly hooked at the ends to which the fibers are attached.

FIGS. 19–29. Pollen mother-cell of *Podophyllum peltatum*. *Figs. 19–24* refer to the first, and *25–29* to the second division.

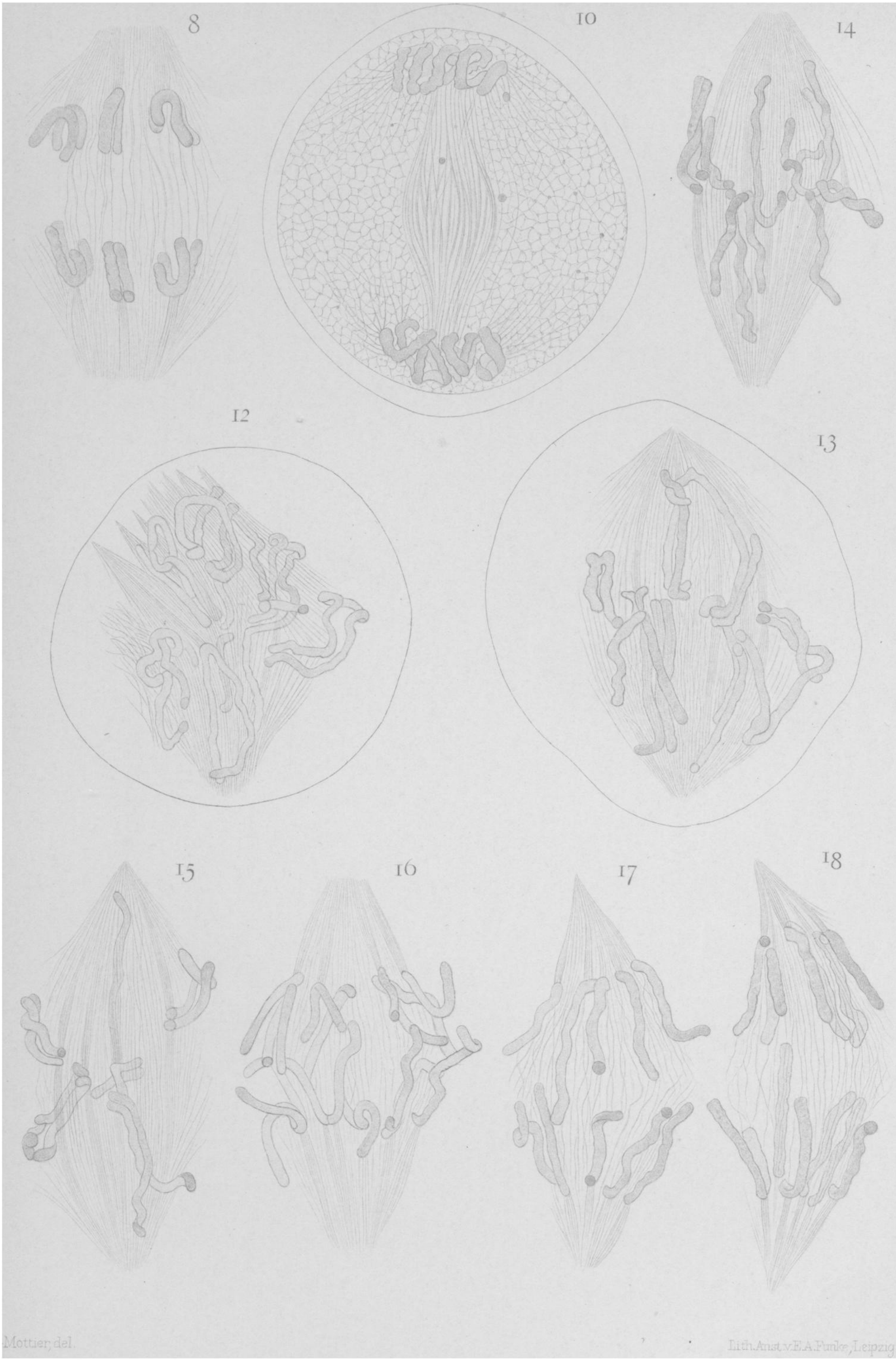
FIGS. 19, 20. Mature spindles; in *fig. 19* the chromosomes on the right and left are ring-shaped; in the central one the segments are in contact at only one end and each is curved; the picture presented by the nuclear plate of *fig. 20* is perhaps more frequent and typical; here also the chromosomes adhere at one end only; the segments are almost invariably curved, and the ends in contact are sometimes fused in such a way as to present a lump or swollen place. × 1500.

FIG. 21, *a* and *b*. Two chromosomes in metakinesis; the second longtudinal fission is plainly manifested; the chromosome *a* was oriented as in *fig. 20*; that at *b* was probably fastened to the spindle by one end, and stood radially to its axis; the retreating daughter chromosomes of *a* will be double U's, while those of *b* will probably give V's, or merely pairs of parallel and contiguous rods. × 1500.

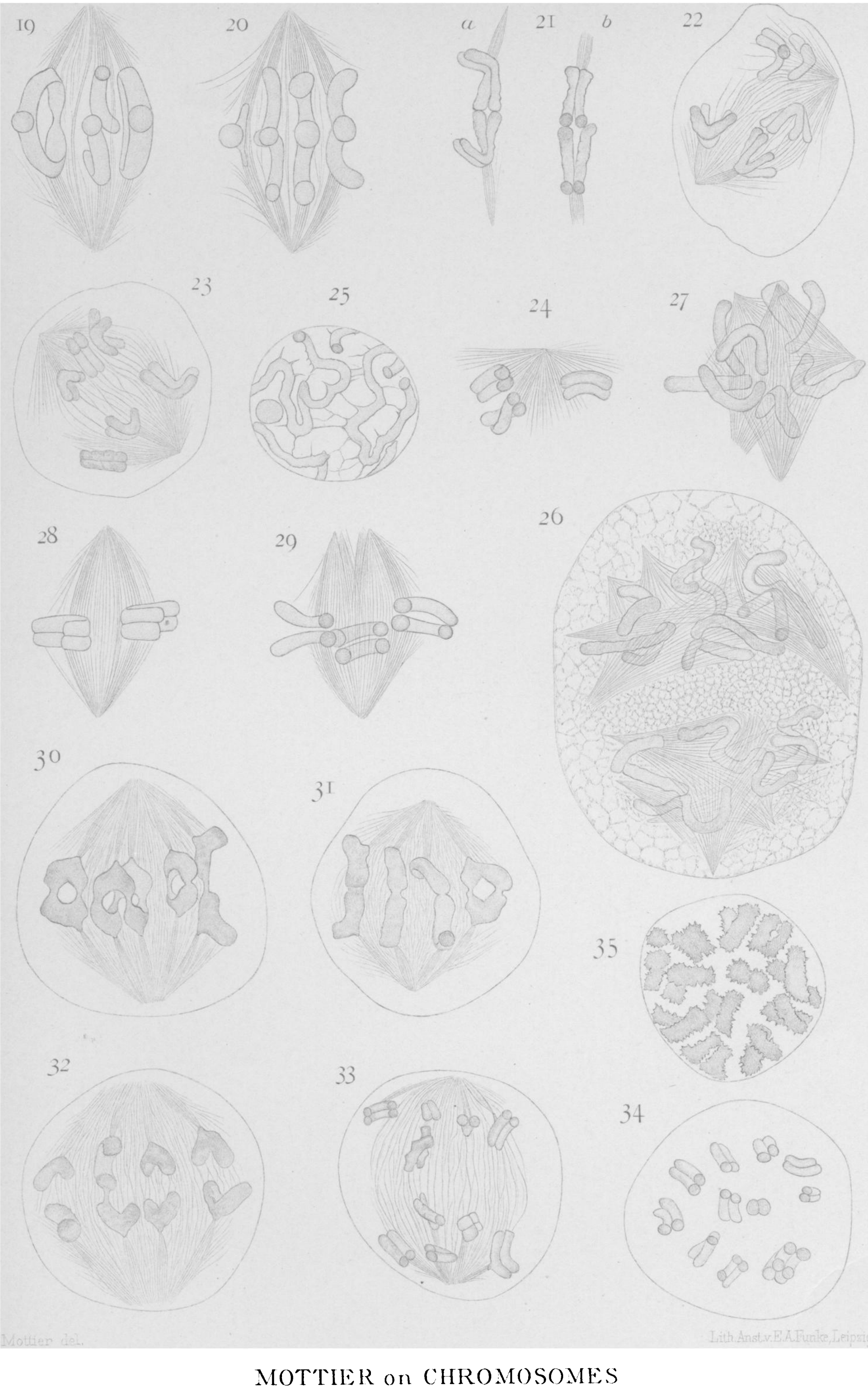
FIGS. 22, 23. Two karyokinetic figures in anaphase; the two granddaughter segments of each daughter chromosome are clearly seen at this stage; they are either somewhat U-shaped, straight, or slightly curved rods. × 1250.



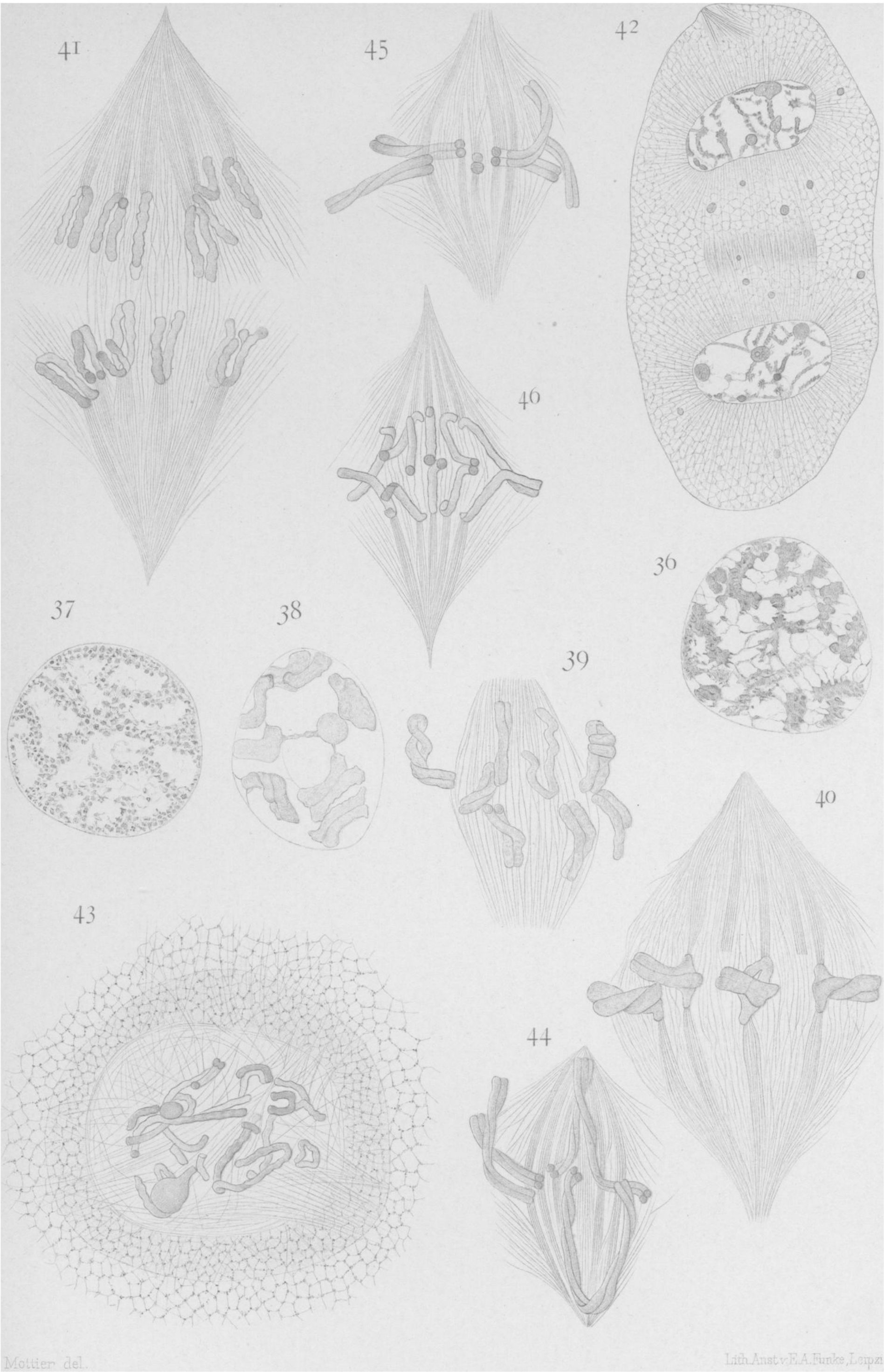
MOTTIER on CHROMOSOMES.



MOTTIER on CHROMOSOMES.



MOTTIER on CHROMOSOMES



MOTTIER on CHROMOSOMES.

FIG. 24. Three chromosomes at a little later stage seen obliquely from the pole. × 1250.

FIG. 25. Daughter nucleus showing hollow spirem; the free ends indicate where the spirem was cut by the microtome knife.

FIG. 26. An entire pollen mother-cell showing the two multipolar spindles of the daughter nuclei. × 1750.

FIG. 27. A multipolar spindle of a daughter nucleus at a later stage than the preceding, in which fewer poles are present; the chromosomes are being arranged in the nuclear plate.

FIGS. 28, 29. Two mature spindles; although in *fig. 29* the segments of the chromosomes are not closely applied to each other, yet this is not a later stage than *fig. 28*.

FIGS. 30–39. *Tradescantia virginica.*

FIG. 30. Mature spindle; the points of the crescent-shaped daughter segments adhere to form ring-like chromosomes except in the one at the right; two of the chromosomes are fastened together by the fusion of one of the points of each crescent to one of the other; in the chromosome at the right only one point of the segments adhere. × 1250.

FIG. 31. The same stage as *fig. 30;* the daughter segments of the three chromosomes on the left are united only at one end; they are short, thick crescents with blunt ends, and when thus oriented they frequently reach almost from pole to pole. × 1250.

FIG. 32. The daughter segments have just separated; at this stage the second longitudinal fission is often not apparent. × 1250.

FIG. 33. The anaphase; at this stage it is evident that the daughter chromosomes have split lengthwise. × 1250.

FIG. 34. The same stage as *fig. 33;* the chromosomes are seen from the pole. × 1250.

FIG. 35. Daughter nucleus seen from the pole: the granddaughter segments of the twelve chromosomes show a tendency to become separated; their surfaces present an eroded appearance. × 1750.

FIG. 36. Daughter nucleus in which the chromosomes are much reticulated and the identity of the individuals cannot be definitely made out. × 1750.

FIG. 37. Daughter nucleus in which all identity of the chromosomes is lost. × 1750.

FIG. 38. The chromosomes are differentiated preparatory to the formation of the spindle. × 1750.

FIG. 39. Mature spindle of second mitosis; the chromosomes are longer and more regular than is usual. × 1750.

FIGS. 40–46. Embryo-sac of *Lilium Martagon.*

FIG. 40. Mature spindle of first karyokinesis in embryo-sac; the chromosomes stand radially upon the spindle. × 1500.

FIG. 41. The anaphase of the same division; the pairs of granddaughter segments are similar to those in the pollen mother-cells.

FIG. 42. Embryo-sac with two daughter nuclei which are nearly in the resting condition; the connecting fibers have not entirely disappeared; less highly magnified.

FIG. 43. An upper daughter nucleus in an early stage of spindle-formation; the nuclear membrane as such has disappeared, and numerous spindle fibers have penetrated the nuclear cavity; the chromatin spirem is not segmented; the free ends have been made by the knife. $\times$ 1500.

FIG. 44. Bipolar spindle of second division; the spirem does not seem completely segmented. $\times$ 1500.

FIG. 45. A mature spindle; the chromosomes are regularly oriented in the nuclear plate; the long, slender segments are contiguous and twisted upon each other. $\times$ 1500.

FIG. 46. The same in metakinesis. $\times$ 1500.